Enhanced Genetic Modification of Adult Growth Factor Mobilized Peripheral Blood Hematopoietic Stem and Progenitor Cells With Rapamycin.

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Public Summary:
The introduction of HIV-resistance genes into blood forming stem cells using lentiviral vectors results in the generation of HIV-resistant (progeny) CD4+ T-cells and monocytes. To date, this process has been fairly inefficient, resulting in less than 1 percent HIV resistant blood cells in patients treated with gene-modified stem cells. We report here on the enhancement of genetic modification of blood forming stem cells using treatment with a widely used drug, rapamycin. We tested the ability of rapamycin to enhance genetic modification of blood forming stem cells using lentiviral vectors encoding HIV-resistance genes. Cells from 9 healthy donors were modified in the presence of rapamycin and demonstrated a 2-3 fold increase in genetic modification compared to those cells modified in the absence of rapamycin. The process was robust and worked for every lentiviral vector and healthy donor sample we tested. We used transplantation of immunodeficient mice to evaluate the ability of rapamycin-treated cells to engraft and produce multiple lineages of blood cells expressing the anti-HIV genes. All mice engrafted and showed an increase in the level of multiple lineages of blood cells (including CD4+ T-cells and monocytes) expressing the introduced gene sequences compared to animals engrafted with cells not treated with rapamycin. There were no safety issues observed in these experiments and the gene modified cells persisted for long periods (>16 weeks) in these mice. These data demonstrate that enhanced transduction of blood forming stem cells results in increased levels of gene-modified blood cells following transplantation of treated rapamycin-treated stem cells. We are currently testing these cells in our clinical trials for HIV gene therapy. These techniques are also useful for other types of gene therapy that utilize lentiviral vectors to genetically modify blood stem cells.

Scientific Abstract:
Genetic modification of adult human hematopoietic stem and progenitor cells (HSPCs) with lentiviral vectors leads to long-term gene expression in the progeny of the HSPCs and has been used to successfully treat several monogenic diseases. In some cases, the gene-modified cells have a selective growth advantage over nonmodified cells and eventually are the dominant engrafted population. However, in disease indications for which the gene-modified cells do not have a selective advantage, optimizing transduction of HSPC is paramount to successful stem cell-based gene therapy. We demonstrate here that transduction of adult CD34+ HSPCs with lentiviral vectors in the presence of rapamycin, a widely used mTORC1 inhibitor, results in an approximately threefold increase in stable gene marking with minimal effects on HSPC growth and differentiation. Using this approach, we have demonstrated that we can enhance the frequency of gene-modified HSPCs that give rise to clonogenic progeny in vitro without excessive increases in the number of vector copies per cell or changes in integration pattern. The genetic marking of HSPCs and expression of transgenes is durable, and transplantation of gene-modified HSPCs into immunodeficient mice results in high levels of gene marking of the lymphoid and myeloid progeny in vivo. The prior safe clinical history of rapamycin in other applications supports the use of this compound to generate gene-modified autologous HSPCs for our HIV gene therapy clinical trials.